JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effects of Wheat Origin, Genotype, and Their Interaction on Multielement Fingerprints for Geographical Traceability

Haiyan Zhao, Boli Guo, Yimin Wei,* and Bo Zhang

Institute of Agro-Products Processing Science and Technology, Chinese Academy of Agricultural Sciences/Key Laboratory of Agro-Products Processing, Ministry of Agriculture, P.O. Box 5109, Beijing 100193, People's Republic of China

ABSTRACT: The objective of this study was to investigate the effects of origin, genotype, and their interaction on multielement fingerprints in wheat kernels to provide theoretical basis for geographical traceability. Ten varieties were grown in three different regions of China during the 2010−2011 growing seasons. The concentrations of 10 elements (Na, Mg, Ca, V, Mn, Fe, Cu, Zn, Mo, and Ba) were determined in 90 wheat kernel samples and 30 provenance soil samples. Multiway analysis of variance results demonstrated that both origin and genotype had significant influences on the content of each element, and their interaction had significant influences on the contents of Mn, Fe, Cu, Zn, Mo, and Ba. The elements Na, Ca, Fe, Zn, and Mo were associated with origin, and Mg, Mn, Cu, and Ba were related to genotype. Na, Ca, Fe, Zn, and Mo were proved to be good indicators to discriminate wheat geographical origin.

KEYWORDS: *traceability, multielement analysis, wheat kernel, geographical origin, genotype*

INTRODUCTION

Food authenticity is receiving increasing interest because the provenance of some foods is regarded as an additional warranty of their quality. Unfortunately, with the increasing mobility and low transport costs of goods existing nowadays, the risk of mislabeling in products with a certified origin should be taken into account. Development of objective and robust analytical methods capable of ensuring geographical traceability is indeed essential and plays a key role in modern food safety control and verification systems, preventing fraud and contributing to consumer confidence in food quality.

Analysis of the elemental composition is an attractive method because elements are stable and may be stored for years without the elemental composition changing significantly. Elemental fingerprints are based on several environmental and geologic factors, such as soil type, rainfall, and temperature of the growing region, and provide a scientific underpinning to determine food geographical origin.[1−3] Creating a fingerprint or unique signature using multielement chemical profiling is an efficient and cost-effective approach to determine the geographical growing region of foods. Elemental composition analysis has been successfully applied to discriminate geographical origins of various products such as wine, tea, virgin olive oil, rice, and onions and other food products.[4−10] The technique is by no means simple, because many factors may influence food element composition and limit the usefulness of the fingerprinting procedure.[11−13] Genotype is one of the main factors influencing the elemental composition of food.[11,13] The key to the successful application of the multielement fingerprinting technique is the selection of suitable elements that would reflect the link with geographical origin and thus have discriminating potential for that particular product.

Wheat is one of the main food crops produced in China and in the world. In a previous study, 240 wheat samples, randomly collected from four different regions in China for two years without considering the effect of genotype, were analyzed by using the multielement fingerprinting technique. It was found that the multielement analysis is a promising method which provided reliable information about wheat origin. The concentrations of 10 elements (Na, Mg, Ca, V, Mn, Fe, Cu, Zn, Mo, and Ba) were significantly different among wheat kernels from different origins.[14] However, most wheat varieties were different in different regions due to differences in climate. The issues that the differences of these 10 element contents among wheat kernels from different origins are caused by the variance of origin or genotype, and whether characteristic fingerprints will change with different genotypes in the same origin are not yet clear. Therefore, it is necessary to study the effects of origin, genotype, and their interaction of wheat on multielement fingerprints to provide theoretical support for identifying food geographical origin by multielement fingerprinting technique. However, as far as we know, no study has been previously published on this aspect.

The objective of this study was to investigate the influences of origin, genotype, and their interaction on wheat multielement fingerprints, and, furthermore, to try to get information about the contributions of each factor to content variance of each element and the elements that are related to geographical origin for traceability.

MATERIALS AND METHODS

Reagents. Ultrapure water (18.5 MΩ·cm) obtained from Milli-Q Millipore system (Bedford, MA, USA) was used for sample pretreatment and dilution. HNO_3, HF, and $HClO_4$ were of metal oxide semiconductor (MOS) grade and from Beijing Institute of Chemical Reagents (Beijing, China). The certified reference materials (CRMs) of wheat flour (GBW10011) and soil (GBW07401), the

Received: May 15, 2012
Revised: October 11, 2012
Accepted: October 12, 2012
Published: October 12, 2012

dx.doi.org/10.1021/jf3021283 | *J. Agric. Food Chem.* 2012, 60, 10957−10962

Table 1. Localization, Weather Conditions, and Growing Periods of Wheat in the Different Sampling Regions

region	N latitude (deg)	E longitude (deg)	altitude (m)	av temp (°C)	total precip (mm)	total sunshine (h)	growth period
Zhaoxian	37.83	114.82	39	7.8	44.9	2926	Oct 5, 2010−June 14, 2011
Huixian	35.39	113.83	82	9.8	65.0	2747	Oct 14, 2010−June 9, 2011
Yangling	34.29	108.06	513	9.4	88.6	2620	Oct 23, 2010−June 6, 2011

internal standard (Rh), and external standards were from the National Research Center for Certified Reference Materials (Beijing, China).

Experimental Design. Field experiments were conducted at three different geographical locations in China: Zhaoxian (Hebei province), Huixian (Henan province), and Yangling (Shaanxi province). Ten wheat varieties (Han 6172, Heng 5229, Hengguan 35, Xinong 889, Xinong 979, Xiaoyan 22, Xinmai 18, Zhengmai 366, Zhoumai 16, and Zhoumai 18) were cultivated on each of three agricultural fields during the 2010 and 2011 growing seasons. The typical size of the plot (variety) was 10 m^2. The experimental design consisted of randomized complete blocks for a total of 10 plots in each station with no replication. At the three locations, the locally recommended agricultural practices regarding seeding rates, fertilization, irrigation, and chemical control of weeds, pests, and diseases were applied to the trials.

Sampling. In each plot, wheat samples were collected in three different sites. At each site, a quadrant of 1 m^2 was randomly selected. A wheat sample was collected at the same time by hand-cutting from the marked quadrant area. The sample was subsequently threshed, and the resulting kernel sample was retained for laboratory analysis. A total of 30 wheat kernel samples were collected from each region.

Soil samples (0−20 cm) were collected from one of the three sites in each plot after wheat harvesting with a soil auger (approximately 500 g in weight for a drill). A total of 10 soil samples were collected from each region.

The localization, meteorological data, and growing periods of wheat for the three selected regions are shown in Table 1. The localization information of the sampling stations was recorded using a portable global positioning system receiver. Meteorological data were collected by automatic weather station for the three regions.

Sample Pretreatment. The wheat kernel samples were thoroughly washed with deionized water to remove surface contamination and then dried in an oven (DHG-9140A, Yiheng, China) at 38 °C for 10 h, until they reached constant weight. To obtain wholemeal, wheat kernel samples were ground in a Cyclotec 1093 sample mill (Foss Tecator, Denmark). Meanwhile, soil samples were air-dried and then crushed to pass through a 0.075 mm nylon mesh sieve and stored in paper bags.

Sample Digestion for ICP-MS. The method for each whole wheat flour sample digestion was as follows: 0.2 g of whole wheat flour was weighed into a 25 mL Teflon vessel; 0.5 mL of water was added to the vessel followed by gentle shaking to obtain homogeneous dispersion. The vessel was capped after the addition of 15 mL of concentrated HNO_3 and then heated over a hot plate (LabTech, USA) at approximately 200 °C for 24 h. After the sample had been resolved, the reactor was opened and the digested solution was evaporated to near dryness on the hot plate. After cooling, the sample digest was dissolved by 4% (v/v) HNO_3, transferred into a 10 mL volumetric flask, and brought to volume with 4% (v/v) HNO_3. The CRM digestion of wheat flour (GBW10011) was performed as above. The obtained digestion solution was used for element determination.

The method for each soil sample digestion was as follows: 0.05 g of soil was weighed into a 25 mL Teflon vessel; 0.5 mL of water was added to the vessel followed by gentle shaking to obtain homogeneous dispersion. The vessel was capped after the addition of 3 mL of concentrated HF, 1 mL of concentrated HNO_3, and 5 drops of concentrated $HClO_4$ and then heated over a hot plate at around 200 °C for 48 h. After the sample had been resolved, the reactor was opened and the digested solution was evaporated to near dryness on the hot plate. After cooling, the residue was dissolved by 4% (v/v) HNO_3, transferred into a 50 mL volumetric flask, and made up to volume with 4% (v/v) HNO_3. The CRM digestion of soil (GBW07401) was performed as above. The obtained digestion solution was used for element determination.

Pressing Pellets for XRF. Soil samples were prepared in the form of pressed powder pellets. For each soil sample, approximately 6 g of soil powder was pressed into a 35 mm internal diameter pellet using a tablet machine (PP40, Retsch, Germany) operated at a pressure of 25 tons for 25 s.

Element Analysis. The concentrations of 10 isotopes (^{23}Na, ^{24}Mg, ^{44}Ca, ^{51}V, ^{55}Mn, ^{57}Fe, ^{65}Cu, ^{66}Zn, ^{97}Mo, and ^{137}Ba) in wheat kernels and five isotopes (^{51}V, ^{65}Cu, ^{66}Zn, ^{97}Mo, and ^{137}Ba) in soil samples were determined using a high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) (Finnigan MAT ELEMENT I, Germany). Optimization was done for maximum sensitivity while maintaining the oxide ratio as low as possible. The operating conditions of the HR-ICP-MS equipment were as follows: 1321 W radio frequency power, 13.88 L·min^{-1} cooling gas flow rate, 0.98 L·min^{-1} auxiliary gas flow rate, 0.524 L·min^{-1} sample gas flow rate, and the nebulization chamber temperature was room temperature (24 °C). For data acquisition the HR-ICP-MS was operated in peak jump mode, and 10 points per peak were used.

For HR-ICP-MS analysis, an online internal standard solution of 0.01 μg·g^{-1} ^{103}Rh was used to compensate for matrix suppression and signal drift during analysis. External standards used for calibration were regularly reinjected after every 10 samples to monitor possible shift of initial calibration. The CRMs of wheat flour (GBW10011) and soil (GBW07401) were analyzed by the proposed methodology to ensure the accuracy of the whole procedure. All sample analyses were carried out in three replicates, and data were expressed as dry matter to correct for the effect of different sample moisture.

The elements Na, Mg, Ca, Mn, and Fe in soil were analyzed using an X-ray fluorescence (XRF) spectrometer (PW2404, Philips, The Netherlands) according to GB/T 14506.28-93.[15] Element concentrations expressed as oxides were converted to element concentrations for statistical analyses.

For XRF analysis, the quality control was performed according to GB/T 14506.28-93.[15] The CRM (GBW07401) was analyzed by the proposed methodology to ensure the accuracy of the whole procedure.

Statistical Analyses. The statistical methods, including one-way analysis of variance (one-way ANOVA), multiway analysis of variance (multiway ANOVA), and linear discriminant analysis (LDA), were carried out with SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA).

One-Way ANOVA. In a first analysis, the emphasis was on detecting significant differences among the tested origins and/or genotypes. One-way ANOVA was applied to the 10 elements employed in this study to test whether the differences in average elemental values relative to considered geographical origins or genotypes were significant with a 5% family significance level. Duncan's multiple comparison following one-way ANOVA was used to identify significant differences between treatments.

Multiway ANOVA. The aim of the second analysis was to quantify the contributions of origin, genotype, and their interaction to the total variance in element levels. Components of variance on the basis of a mixed model were computed considering origin and genotype as fixed. The contributions of origin, genotype, and their interaction to the observed variability were calculated by the variance component ratio $\sigma_i^2/\sigma_{total}^2$ (σ_i^2 = square sum of variance of certain factor; σ_{total}^2 = total square sum of variance). A larger ratio indicates the greater influence and stability of a certain factor relative to the variability.

LDA. LDA is a supervised pattern recognition, which means that the class membership has to be known prior to the analysis. The groups to be discriminated can be defined naturally by the problem under investigation. LDA maximizes the variance between groups and

Table 2. Results Obtained through the Analysis of Wheat and Soil CRM[a]

element	CRM ($\mu g \cdot g^{-1}$)	exptl value ($\mu g \cdot g^{-1}$)	recovery (%)	element	CRM ($\mu g \cdot g^{-1}$)	exptl value ($\mu g \cdot g^{-1}$)	recovery (%)
(A) Wheat CRM							
Na	17 ± 5	16.6 ± 0.7	98 ± 4	Fe	18.5 ± 3.1	17.7 ± 0.6	95 ± 3
Mg	450 ± 70	445 ± 15	99 ± 3	Cu	2.7 ± 0.2	2.6 ± 0.2	95 ± 6
Ca	340 ± 20	340 ± 12	100 ± 4	Zn	11.6 ± 0.7	10.9 ± 0.9	94 ± 8
V	0.034 ± 0.012	0.031 ± 0.001	91 ± 3	Mo	0.48 ± 0.05	0.44 ± 0.02	91 ± 5
Mn	5.4 ± 0.3	5.3 ± 0.2	98 ± 4	Ba	2.4 ± 0.3	2.2 ± 0.2	93 ± 6
(B) Soil CRM							
Na	450 ± 100	442 ± 29	98 ± 6	Fe	22500 ± 500	22396 ± 562	100 ± 2
Mg	490 ± 120	504 ± 23	103 ± 5	Cu	19 ± 2	19.4 ± 1.2	102 ± 6
Ca	2130 ± 280	2121 ± 107	100 ± 5	Zn	20 ± 2	19.3 ± 1.3	96 ± 6
V	33 ± 3	31 ± 2	95 ± 5	Mo	0.76 ± 0.14	0.702 ± 0.016	92 ± 2
Mn	155 ± 7	153 ± 13	98 ± 8	Ba	143 ± 14	134 ± 6.6	94 ± 5

[a] The experimental values in this table are shown as the mean ± standard deviation.

minimizes the variance within the group by creating new variables (discriminant functions), which are linear combinations of the original variables (elemental concentrations) and equal to the number of categories minus 1. The robustness of the model was demonstrated by cross-validation, where each time one sample was omitted from the model and classified by new functions derived from the remaining samples. The separation among groups in the discriminant space was checked by plotting the first two function scores.

RESULTS

Validation of the Proposed Method. The certified values showed good agreement with measured values (Table 2). The mean recoveries of the 10 elements were between 90 and 105%, with standard deviations of <10%. The results were satisfactory, and the proposed methodology was used for the analysis of the samples.

One-Way ANOVA. The concentrations of all elements are presented as the mean and standard deviation (SD) for each of the categories tested (Tables 3−5). Statistically significant differences in the concentrations of all measured elements were observed in wheat samples from the three geographical regions (Table 3). The regions were characterized by different element combinations. Wheat kernels grown in Zhaoxian were enriched in Mg, V, Cu, and Mo. The mean concentrations of Zn and Ba in the samples were highest and Na and Ca were lowest in Huixian. The samples from Yangling were characterized by the highest mean contents of Na, Ca, Mn, and Fe and the lowest Zn mean content.

Table 3. Element Contents (Micrograms per Gram) in Wheat Samples from Different Origins[a]

element	Zhaoxian	Huixian	Yangling
Na	26.2 ± 2.8 b	23.8 ± 2.4 c	28.6 ± 3.3 a
Mg	1410 ± 179 a	1206 ± 153 b	1322 ± 205 a
Ca	461 ± 39 b	433 ± 40 c	505 ± 39 a
V	0.031 ± 0.015 a	0.024 ± 0.019 ab	0.022 ± 0.010 b
Mn	32.6 ± 4.0 b	31.7 ± 2.8 b	36.9 ± 3.5 a
Fe	48.2 ± 6.8 b	48.5 ± 5.5 b	60.3 ± 6.8 a
Cu	4.27 ± 0.72 a	3.77 ± 0.72 b	4.06 ± 0.73 ab
Zn	24.1 ± 4.2 b	31.6 ± 4.8 a	19.4 ± 3.0 c
Mo	0.609 ± 0.127 a	0.415 ± 0.084 b	0.391 ± 0.078 b
Ba	3.68 ± 0.94 a	3.98 ± 1.06 a	3.14 ± 0.70 b

[a] Data are shown as the mean ± standard deviation, and the different letters indicate significant differences ($p < 0.05$).

In relation with the wheat varieties, significant differences were observed in the concentrations of the different elements with the exception of Zn (Table 4). Each variety had its own elemental profile. The mean concentrations of Ca and Ba were highest and Mo was lowest in Han 6172; Mg was lowest in Hengguan 35; Ca was lowest in Xiaoyan 22; Mg, V, Mn, Fe, and Mo were highest in Xinmai 18; Cu was highest in Xinong 889; Na was highest in Xinong 979; Na and Ba were lowest in Zhengmai 366; Mn, Fe, and Cu were lowest in Zhoumai 16; and V was lowest in Zhoumai 18.

There were significant differences among different regions for the 10 element contents in soil samples (Table 5). Soil samples from different regions had their own elemental characteristics. The mean contents of Na and Mg were highest and V, Mn, Fe, Cu, Zn, Mo, and Ba were lowest in Zhaoxian soil; Na, Mg, and Ca were lowest in Huixian soil; and Ca, V, Mn, Fe, Cu, Zn, Mo, and Ba were highest in Yangling soil.

Multiway ANOVA. To select the elements closely related to geographical origin, the effects of origin, genotype, and their interaction were compared by means of multiway ANOVA test. The concentrations of elements in kernel as a whole were extremely significantly influenced ($p < 0.001$) by origin, genotype, and the interaction of origin and genotype. The influence order of each factor was origin > genotype > interaction (Wilkes' λ gave an F_{origin} value of 85.7, an F_{genotype} of 9.75, and an $F_{\text{interaction}}$ of 2.50). Specifically, both origin and genotype had significant influences on the content of each element, and their interaction had significant influences on the contents of Mn, Fe, Cu, Zn, Mo, and Ba ($p < 0.05$). Origin percentages of total square sum of content variance of Na, Mg, Ca, V, Mn, Fe, Cu, Zn, Mo, and Ba were 33.2, 18.2, 36.7, 5.79, 30.6, 44.3, 7.8, 61.4, 50.2, and 13.2%, respectively. Genotype percentages of total square sum were 27.8, 40.8, 26.8, 23.9, 31.5, 22.8, 58.6, 10.8, 30.7, and 54.2%, respectively. Those of their interaction were 10.1, 13.7, 12.0, 19.7, 17.8, 13.2, 14.0, 16.3, 10.7, and 14.5%, respectively. The contributions of error to total variances were 28.9, 27.3, 24.5, 50.7, 20.1, 19.6, 19.6, 11.6, 8.5, and 18.1%, respectively. The origin effect was observed to be strongest on Na, Ca, Fe, Zn, and Mo content variabilities. Genotype effect was strongest on Mg, Mn, Cu, and Ba content variabilities. V content variability was mainly affected by error.

LDA. LDA was used to evaluate whether the elements Na, Ca, Fe, Zn, and Mo associated with geographical origin enable fingerprinting of wheat kernels and their classification according to geographical origin. Two canonical discriminant functions were derived on the basis of these five elements associated with

Table 4. Element Contents (Micrograms per Gram) in Wheat Samples from Different Varieties[a]

element	Han 6172	Heng 5229	Hengguan 35	Xiaoyan 22	Xinmai 18	Xinong 889	Xinong 979	Zhengmai 366	Zhoumai 16	Zhoumai 18
Na	25.8 ± 2.1 b	25.0 ± 4.4 b	25.9 ± 2.6 b	25.7 ± 2.7 b	26.5 ± 2.7 b	27.1 ± 3.2 b	31.1 ± 3.9 a	24.7 ± 2.6 b	25.1 ± 2.3 b	24.9 ± 3.3 b
Mg	1331 ± 180 b	1273 ± 240 b	1203 ± 164 b	1225 ± 169 b	1662 ± 188 a	1313 ± 130 b	1265 ± 71 b	1361 ± 142 b	1240 ± 163 b	1253 ± 84 b
Ca	501 ± 58 a	441 ± 46 c	455 ± 44 abc	436 ± 39 c	499 ± 58 a	493 ± 20 ab	490 ± 48 ab	438 ± 55 c	459 ± 19 abc	448 ± 35 bc
V	0.019 ± 0.003 bc	0.037 ± 0.028 a	0.022 ± 0.005 bc	0.024 ± 0.008 abc	0.038 ± 0.027 a	0.034 ± 0.016 ab	0.024 ± 0.008 abc	0.020 ± 0.004 bc	0.019 ± 0.006 bc	0.018 ± 0.005 c
Mn	35.4 ± 3.6 ab	34.9 ± 3.2 ab	31.8 ± 2.7 bc	31.9 ± 3.6 bc	36.5 ± 4.1 a	35.3 ± 1.4 ab	30.9 ± 4.0 c	35.9 ± 4.3 a	29.6 ± 4.1 c	35.0 ± 3.9 ab
Fe	54.3 ± 10.0abc	53.4 ± 8.4 abc	48.8 ± 7.8 bc	48.8 ± 5.1 bc	59.7 ± 9.1 a	56.4 ± 5.4 ab	51.4 ± 9.3 abc	55.6 ± 8.8 ab	46.9 ± 7.7 c	48.0 ± 5.0 bc
Cu	4.51 ± 0.62 ab	4.08 ± 0.45 bc	3.66 ± 0.49 cd	3.46 ± 0.45 de	4.54 ± 0.52 ab	4.94 ± 0.78 a	4.39 ± 0.36 b	4.23 ± 0.43 b	3.04 ± 0.33 e	3.49 ± 0.47 de
Zn	26.0 ± 2.4 a	24.7 ± 6.9 a	23.9 ± 5.4 a	23.8 ± 6.7 a	28.2 ± 9.0 a	25.0 ± 7.0 a	22.5 ± 4.2 a	26.0 ± 8.1 a	21.6 ± 5.7 a	28.5 ± 6.4 a
Mo	0.35 ± 0.11 d	0.48 ± 0.19 abcd	0.41 ± 0.10bcd	0.39 ± 0.08 cd	0.60 ± 0.14 a	0.51 ± 0.07abc	0.47 ± 0.09 abcd	0.57 ± 0.20 a	0.41 ± 0.08bcd	0.53 ± 0.10 ab
Ba	5.02 ± 0.97 a	3.60 ± 0.78 cd	3.15 ± 0.56 def	2.70 ± 0.37 ef	3.64 ± 0.76 cd	3.68 ± 0.68bcd	4.05 ± 1.11 bc	2.55 ± 0.14 f	3.26 ± 0.48 de	4.35 ± 0.51 b

[a] Data are shown as the mean ± standard deviation, and the different letters indicate significant differences ($p < 0.05$).

Table 5. Element Contents (Micrograms per Gram) in Soil Samples from Different Origins[a]

element	Zhaoxian	Huixian	Yangling
Na	13712 ± 239 a	9983 ± 219 c	11278 ± 412 b
Mg	13428 ± 294 a	10716 ± 76 c	12612 ± 141 b
Ca	28414 ± 333 b	23352 ± 318 c	41663 ± 2528 a
V	78.4 ± 3.5 c	92.0 ± 3.0 b	100 ± 3 a
Mn	516 ± 26 c	613 ± 12 b	742 ± 17 a
Fe	28014 ± 1165 c	32445 ± 419 b	34755 ± 286 a
Cu	24.6 ± 1.3 b	30.4 ± 1.0 a	31.3 ± 1.2 a
Zn	66.8 ± 13.4 b	71.2 ± 2.8 ab	75.5 ± 3.0 a
Mo	0.45 ± 0.07 c	0.71 ± 0.10 b	0.86 ± 0.15 a
Ba	518 ± 21 b	535 ± 11 b	577 ± 25 a

[a] Data are shown as the mean ± standard deviation, and the different letters indicate significant differences ($p < 0.05$).

origin. Na, Fe, and Zn were the variables with the higher weights in discriminant function 1. Discriminant function 2 was formed mainly by Ca and Mo. The total correct classification of 91.1% was achieved by the two discriminant functions with cross-validation. The validation results are presented in Table 6.

Table 6. Classification of Wheat Samples from Different Regions

		predicted group membership			
		Zhaoxian	Huixian	Yangling	total
		Original			
count	Zhaoxian	28	2	0	30
	Huixian	3	27	0	30
	Yangling	2	0	28	30
%		93.3	90.0	93.3	92.2
		Cross-Validated			
count	Zhaoxian	27	3	0	30
	Huixian	3	27	0	30
	Yangling	2	0	28	30
%		90.0	90.0	93.3	91.1

Between Zhaoxian and Huixian samples, there were some misclassifications in both directions, which indicated that elemental characteristics in wheat kernels from the two regions shared some similarities. There was no misclassification between Huixian and Yangling, suggesting that elemental fingerprints of wheat from the two regions had large differences.

Scores plot (Figure 1) showed good separation between different origins, suggesting that these five elements contained sufficient information to assess the geographical origin of wheat.

■ DISCUSSION

The results of one-way ANOVA showed that the concentrations of the elements Na, Mg, Ca, V, Mn, Fe, Cu, Zn, Mo, and Ba in kernels differed significantly among various regions, which was in good agreement with our previous study.[14] This can be explained by the fact that weather conditions (average temperature, total precipitation, and sunshine hours) during growing periods and soil conditions of the three regions were different (Tables 1 and 5). Significant differences between locations were found for Ba in wheat by Laursen et al., which was consistent with our result.[16] Moreover, for different varieties, the concentrations of the elements Na, Mg, Ca, V,

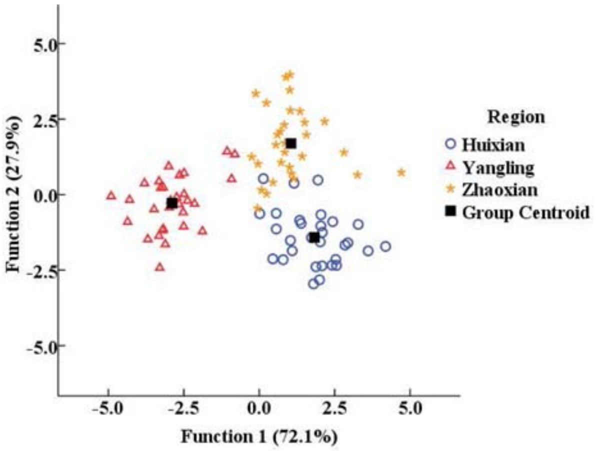

Figure 1. Scatter plot of discriminant functions 1 and 2 of wheat samples.

Mn, Fe, Cu, Mo, and Ba in kernels were significantly different. This indicated that genotype may affect the uptake of elements by wheat. Some studies also found that kernel Fe content showed significant differences among different wheat genotypes.[17,18]

Soil samples from different origins had their own elemental characteristics. This could be explained by the fact that the soil types were different among the three regions. The soil types of Zhaoxian, Huixian, and Yangling are loam, clay, and brunisolic soils, respectively. Zhao and Jin indicated that the content of Ca in soil of China increases from southeast to northwest.[19] Zheng et al. showed that the contents of V, Cu, and Zn in soil of China gradually decrease from south to north and that of Mn gradually decreases from west to east.[20] Dong and Sun found that the content of Fe in soil of Shaanxi province is highest and Hebei province is lowest among these three provinces.[21] These are consistent with our results. No published literature has been found on the contents of Na, Mg, Mo, and Ba in soils of these three regions.

The results of multiway ANOVA showed that both geographical origin and genotype had significant influences on the content of each element, and their interaction had significant influences on the contents of Mn, Fe, Cu, Zn, Mo, and Ba. The influence of geographical origin on Na, Ca, Fe, Zn, and Mo content variabilities in wheat was highest. It could be seen from Tables 3 and 5 that the mean content of Ca in soil was highest in Yangling and lowest in Huixian; the mean content of Fe in soil was highest in Yangling and lowest in Zhaoxian. They were in line with those in kernels. The mean content of Mo in soil was highest in Yangling and lowest in Zhaoxian, whereas that in kernel was just the opposite. These results indicated that the contents of Ca, Fe, and Mo in kernels were mainly associated with soil provenance. The contents of Ca and Fe in wheat kernels increased and Mo decreased with increasing of respective contents in soil. However, the contents of Na and Zn in soil were not quite in line with those in wheat, which could be explained by the fact that the origin factor involves not only soil but climate and agricultural practices and so on. This suggested that the contents of Na and Zn in wheat were mainly influenced by climate, agricultural practices, or other factors related to origin, but not soil. The genotype effect was mainly observed for Mg, Mn, Cu, and Ba content variabilities, which indicated that the uptake of Mg, Mn, Cu, and Ba by wheat was under genetic control. The content of V in wheat kernel was relatively low, which could explain why V content variability in kernel was mainly affected by error.

There have been some reports about the effects of origin, genotype, and their interaction on mineral contents in wheat kernels, which are consistent with our results.[11,13,22−25] Ficco et al. revealed that Na content in kernel was most influenced by environment.[22] Peterson et al. determined Mg, Ca, Fe, and Zn concentrations of 27 wheat cultivars from six locations. The concentrations of these elements in kernels were highly influenced ($p < 0.01$) by production site, cultivar differences, and the interaction of location and cultivar effects. The effects of production site on Ca, Fe, and Zn are larger than that of genotype, but smaller on Mg variability.[11] Oury et al. used different sources of genotypes grown in different environments to study kernel Mg and Zn concentrations in bread wheat (*Triticum aestivum* L.). They found that significant environmental effects and genotype effects appeared for Mg and Zn concentrations.[23] Zhang et al. found that genotype, environment, and their interaction all had highly significant effects on the concentrations of Mn, Fe, Cu, and Zn.[13] Joshi et al. showed that the effects of environment and the interaction of environment and genotype have significant influences on Fe and Zn variabilities. Fe and Zn concentrations in wheat kernel depend largely on environmental conditions.[24] The study of Morgounov et al. found that wheat kernel Zn concentration was almost totally dependent on location.[25] Little attention has been given to V, Mo, and Ba uptake by plants.

The elements Na, Ca, Fe, Zn, and Mo were associated with geographical origin, and the model built with these five elements enabled the correct classification of 91.1% of wheat samples from different regions by cross-validation. The successful classification of the samples supported that these five element contents of the tested samples enable fingerprinting of wheat kernels and their classification according to geographical origin. Nevertheless, more samples from the same and other wheat districts must be analyzed to consolidate this conclusion.

It could be found from this study that both origin and genotype had significant influences on content variabilities of Na, Mg, Ca, V, Mn, Fe, Cu, Zn, Mo, and Ba, and their interaction had significant influences on content variabilities of Mn, Fe, Cu, Zn, Mo, and Ba. The origin effect was observed to be strongest on Na, Ca, Fe, Zn, and Mo content variabilities. Genotype effect was strongest on Mg, Mn, Cu, and Ba content variabilities. The elements Na, Ca, Fe, Zn, and Mo were identified as good indicators to discriminate wheat geographical origin. This is an important prerequisite for the application of the fingerprinting methodology and a first result for this kind for food.

■ AUTHOR INFORMATION

Corresponding Author

* E-mail: weiyimin36@126.com. Fax: 0086-10-62895141.

Funding

This project was supported by Special Fund for Basic Scientific Research of Central Public Welfare Research Institutes (No. zwjj2012yyjg02), Special Construction of Industrial Technology System for Wheat (No. CARS-03), National Natural Science Foundation of China (No. 30800862), and Special Fund for Agro-scientific Research in the Public Interest (No. 200903043).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the Beijing Research Institute of Uranium Geology for instrument and technical assistance.

■ REFERENCES

(1) Herawati, N.; Suzuki, S.; Hayashi, K.; Rivai, I. F.; Koyama, H. Cadmium, copper, and zinc levels in rice and soil of Japan, Indonesia, and China by soil type. *Bull. Environ. Contam. Toxicol.* **2000**, *64*, 33−39.

(2) Khoshgoftarmanesh, A. H.; Shariatmadari, H.; Karimian, N.; Kalbasi, M.; van der Zee, S. E. A. T. M. Cadmium and zinc in saline soil solutions and their concentrations in wheat. *Soil Sci. Soc. Am. J.* **2006**, *70*, 582−589.

(3) Purvis, O. W.; Dubbin, W.; Chimonides, P. D. J.; Jones, G. C.; Read, H. The multi-element content of the lichen *Parmelia sulcata*, soil, and oak bark in relation to acidification and climate. *Sci. Total Environ.* **2008**, *390*, 558−568.

(4) Coetzee, P. P.; Steffens, F. E.; Eiselen, R. J.; Augustyn, O. P.; Balcaen, L.; Vanhaecke, F. Multi-element analysis of south African wines by ICP-MS and their classification according to geographical origin. *J. Agric. Food Chem.* **2005**, *53*, 5060−5066.

(5) Pilgrim, T. S.; Watling, R. J.; Grice, K. Application of trace element and stable isotope signatures to determine the provenance of tea (*Camellia sinensis*) samples. *Food Chem.* **2010**, *118*, 921−926.

(6) Benincasa, C.; Lewis, J.; Perri, E.; Sindona, G.; Tagarelli, A. Determination of trace element in Italian virgin olive oils and their characterization according to geographical origin by statistical analysis. *Anal. Chim. Acta* **2007**, *585*, 366−370.

(7) Ariyama, K.; Shinozaki, M.; Kawasaki, A. Determination of the geographic origin of rice by chemometrics with strontium and lead isotope ratios and multielement concentrations. *J. Agric. Food Chem.* **2012**, *60*, 1628−1634.

(8) Gonzálvez, A.; Armenta, S.; de la Guardia, M. Geographical traceability of "Arròs de Valencia" rice grain based on mineral element composition. *Food Chem.* **2011**, *126*, 1254−1260.

(9) Ariyama, K.; Horita, H.; Yasui, A. Application of inorganic element ratios to chemometrics for determination of the geographic origin of welsh onions. *J. Agric. Food Chem.* **2004**, *52*, 5803−5809.

(10) Ariyama, K.; Aoyama, Y.; Mochizuki, A.; Homura, Y.; Kadokura, M.; Yasui, A. Determination of the geographic origin of onions between three main production areas in Japan and other countries by mineral composition. *J. Agric. Food Chem.* **2007**, *55*, 347−354.

(11) Peterson, C. J.; Johnson, V. A.; Mattern, P. J. Influence of cultivar and environment on mineral and protein concentrations of wheat flour, bran and grain. *Cereal Chem.* **1986**, *63*, 183−186.

(12) Perilli, P.; Mitchell, L. G.; Grant, C. A.; Pisante, M. Cadmium concentration in durum wheat grain (*Triticum turgidum*) as influenced by nitrogen rate, seeding date and soil type. *J. Sci. Food Agric.* **2010**, *90*, 813−822.

(13) Zhang, Y.; Song, Q.; Yan, J.; Tang, J.; Zhao, R.; Zhang, Y.; He, Z.; Zou, C.; Ortiz-Monasterio, I. Mineral element concentrations in grains of Chinese wheat cultivars. *Euphytica* **2010**, *174*, 303−313.

(14) Zhao, H.; Guo, B.; Wei, Y.; Zhang, B.; Sun, S.; Zhang, L.; Yan, J. Determining the geographic origin of wheat using multielement analysis and multivariate statistics. *J. Agric. Food Chem.* **2011**, *59*, 4397−4402.

(15) The People's Republic of China. Methods for chemical analysis of silicate rocks: silicate rocks − determination of contents of major and minor elements − X-ray fluorescence spectrometric method, 1993.

(16) Laursen, K. H.; Schjoerring, J. K.; Olesen, J. E.; Askegaard, M.; Halekoh, U.; Husted, S. Multielemental fingerprinting as a tool for authentication of organic wheat, barley, faba bean, and potato. *J. Agric. Food Chem.* **2011**, *59*, 4385−4396.

(17) Lu, L.; Ji, Y.; Li, L.; Li, Z.; Wu, Y. Analysis of Fe, Zn and Se contents in different wheat cultivars (lines) planted in different areas. *Chin. J. Appl. Environ. Biol.* **2010**, *16* (5), 646−649.

(18) Zhao, F. J.; Su, Y. H.; Dunham, S. J.; Rakszegi, M.; Bedo, Z.; McGrath, S. P.; Shewry, P. R. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J. Cereal Sci.* **2009**, *49*, 290−295.

(19) Zhao, S. P.; Jin, L. Studies on the localized optimal estimation of calcium content of topsoil in China. *Acta Sci. Circumstantiae* **1992**, *12*, 168−173.

(20) Zheng, C. J.; Zhang, D. W.; Li, H. M.; Wu, D. T. Content and distribution of trace elements of topsoil in China. *Environ. Monit. China* **1992**, *8*, 8−12.

(21) Dong, X. H.; Sun, W. S. Content and distribution of Fe and Al in the soils of China. *Environ. Monit. China* **1991**, *7*, 1−3.

(22) Ficco, D. B. M.; Riefolo, C.; Nicastro, G.; De Simone, V.; Di Gesù, A. M.; Beleggia, R.; Platani, C.; Cattivelli, L.; De Vita, P. Phytate and mineral elements concentration in a collection of Italian durum wheat cultivars. *Field Crop. Res.* **2009**, *111*, 235−242.

(23) Oury, F.-X.; Leenhardt, F.; Rémésy, C.; Chanliaud, E.; Duperrier, B.; Balfourier, F.; Charmet, G. Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. *Eur. J. Agron.* **2006**, *25*, 177−185.

(24) Joshi, A. K.; Crossa, J.; Arun, B.; Chand, R.; Trethowan, R.; Vargas, M.; Ortiz-Monasterio, I. Genotype environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. *Field Crop. Res.* **2010**, *116*, 268−277.

(25) Morgounov, A.; Gómez-Becerra, H. F.; Abugalieva, A.; Dzhunusova, M.; Yessimbekova, M.; Muminjanov, H.; Zelenskiy, Y.; Ozturk, L.; Cakmak, I. Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica* **2007**, *155*, 193−203.